METABOLIC ALTERATIONS IN RATS EXPOSED TO ACUTE ACCELERATION STRESS[1]

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ABSTRACT. Fasted female Sprague-Dawley rats were exposed to 4.5 g for varying periods ranging from 0.5 to 96 hr employing an 8.5-foot radius centrifuge. During the first 24 hr, there was a significant and sustained increase in blood glucose, plasma free fatty acid, and plasma corticosterone. Plasma free amino acids were decreased. Several of the glucogenic amino acids (alanine, arginine, phenylalanine-tyrosine and proline) were decreased preferentially. There was a progressive increase in liver glycogen deposition detectable within the first hr of centrifugation which reached a maximum after 5 to 24 hr. The glycogen response was eliminated by adrenalectomy and hypophysectomy. Adrenodemedullated rats showed a decreased glycogen response. Alloxan-diabetic rats did not show any increase in liver glycogen above the initially high levels present. The combined stresses of centrifugation and starvation effected a marked depletion of both liver and gastrocnemius muscle glycogen. Starvation alone resulted in an increase in liver glycogen with a concomitant fall in muscle glycogen. It is concluded that the liver glycogen deposited in acceleration stressed rats is mediated by the elaboration of adrenocorticosterone following activation of the pituitary-adrenal system.

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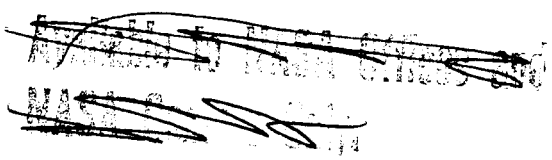
In previous work from this laboratory (1), it was reported that one of the significant stress effects in fasted mice exposed to centrifugal acceleration is a marked increase in liver glycogen deposition. Similar effects have also been noted in animals exposed to other stresses including x-irradiation (2), prolonged starvation (3), and scalding (4). There is much evidence to support the view that this increased liver glycogen deposition is due to the stimulation of adrenocortical function. However, other factors are undoubtedly involved since other stressful stimuli which affect adrenocortical activity, such as severe exercise (5), hypothermia (6), CCl_4 intoxication (7), and transplanted tumors (8), do not lead to an increase in liver glycogen deposition.

This study was undertaken to investigate the metabolic responses of rats subjected to relatively short periods of acceleration stress and also to extend our previous investigation on factors involved in stress induced liver glycogenesis.

Materials and Methods

Animals. Female Sprague-Dawley rats weighing 175-300 g were used in these studies. Animals were housed in an air-conditioned room next to the centrifuge facility. They were maintained on Purina Laboratory Chow and had free access to water. Hypophysectomized (HYPOX), adrenalectomized (ADRX), and adrenodemedullated (ADM) rats were obtained from Simonsen Laboratory, Gilroy, Calif. Completeness of hypophysectomy and adrenalectomy was checked at autopsy by macroscopic examination. HYPOX rats were given 5% glucose and ADRX rats 0.9% saline solution for drinking purposes. Alloxan-diabetes was induced by the intraperitoneal injection of alloxan (200 mg/kg) into 72 hr fasted rats. Insulin (2 U/rat) was



administered daily for the first week only to reduce the mortality rate. The alloxan-diabetic rats were used one month after treatment and only those having overnight fasting blood glucose levels exceeding 350 mg per 100 ml were used in this study.

Centrifuge. A ten radial armed centrifuge having an effective operating radius of 8.5 feet was employed. Animal cages (10×20×10 inches) were suspended from each arm by means of a pivotal yoke assembly. The cages had one degree of freedom and swung outwardly with centrifugation. The position assumed by the cages during centrifugation was such that the resultant force to which the aminals were exposed was normal to the cage floor. All cages were illuminated with individual fluorescent lights automated to provide daily on and off cycling at 6 AM and 6 PM, respectively.

General Procedure. Overnight fasted rats were divided into two groups; one group was placed on the centrifuge while the remaining group served as controls. The combined period of fasting and centrifugation totaled 20-24 hr for all experiments except as otherwise noted. Immediately following centrifugation, both groups of rats were sacrificed by decapitation or by heart puncture under pentobarbital anesthesia. Blood samples were collected in heparinized tubes. Tissue samples from liver and gastrocnemius muscle were taken, weighed, and placed in hot 30% KOH solution within one minute of exsanguination. The glycogen was precipitated with ethanol and measured by the method of Montgomery (9). Blood glucose was measured by the method of Nelson-Somogyi (10); plasma free amino acid (FAA) nitrogen by the method of Rapp (11). Plasma free fatty acids (FFA) were determined by Trout's modification (12) of Dole's

method (13). Plasma corticosterone was measured fluorometrically with a micro modification of Peterson's method (14) as described by Wherry, et al. (15). Analyses of corticosterone by the double isotope derivative method (16) were done under the supervision of Dr. Leo E. Gaudette of the NEN Biomedical Assay Laboratory, Boston, Mass. Quantitative analyses of plasma free amino acids (FAA) were performed on picric acid treated plasma samples using a Beckman-Spinco amino acid analyzer according to the method of Moore, Spackman, and Stein (17).

Results

Figure 1 representing the combined data of three separate experiments shows the progressive increase in liver glycogen deposition of centrifuged rats as a function of exposure time. A maximum increase is effected after 5 to 24 hr of centrifugation. A highly significant increase ($P < 0.001$) is detectable as early as the first hr of centrifugation.

Concomitant changes in blood glucose, plasma FFA, and plasma FAA levels are shown in Fig. 2. Both blood glucose and plasma FFA are increased significantly for all exposure periods. Analyses of plasma FAA by ion-exchange chromatography on pooled samples from rats centrifuged for 2.5 hr along with corresponding controls are shown in Table 1. It can be seen that there is a generalized reduction in plasma FAA; the over-all reduction in the centrifuged rats was 25%. Several of the amino acids including alanine, arginine, phenylalanine-tyrosine, and proline show a more pronounced decrease ranging from 38-47%.

Plasma corticosterone levels in centrifuged rats are increased significantly for all exposure periods (Table 2). Although values for corticosterone by the fluorometric method are considerably higher than

those obtained by the double labeled isotopic method, in general, the relative changes are similar and comparable. In order to minimize the diurnal effects on plasma corticosterone levels (18), all groups of rats were sacrificed between 9-11 AM.

To further delineate the functional role of the adrenocortical system on the glycogen deposition response, centrifuge studies were run on adrenodemedullated (ADM), hypophysectomized (HYPOX), adrenalectomized (ADRX), and alloxan-diabetic rats. ADM rats were employed 4 months after operation; ADRX rats, 5 days; HYPOX rats, 9 days; and alloxan-diabetic rats, 30 days. Glycogen response and corresponding changes in plasma corticosterone of these animals are shown in Table 3. It is observed that compared to the glycogen response of normal intact rats, only that of the ADM rats is significant. The lack of response of alloxan-diabetic rats is undoubtedly related to the exceptionally high levels of glycogen retained by these animals upon overnight fasting. Plasma corticosterone levels are increased significantly in centrifuged normal, ADM, and alloxan-diabetic rats but are virtually unchanged in HYPOX and ADRX rats. Thus, except for the anomalous response of alloxan-diabetic rats, increased liver glycogen deposition is accompanied by a rise in plasma corticosterone.

Table 4 shows the liver glycogen of rats subjected to 4.5 g for 5 hr with one group sacrificed immediately after the exposure and others after a 24 hr period off the centrifuge. The rats taken off the centrifuge were further divided into two groups. One group was continued on a fasting regime while the other group was fed ad lib. After the 24 hr period off the centrifuge both groups were sacrificed and their liver glycogen measured. It can be seen that the glycogen deposited in response to centrifugation is completely lost when rats are fasted for an additional

24 hr. The ability to deposit optimal amounts of glycogen after feeding is not affected by prior exposure to centrifugation. This is evident from the amount of glycogen deposited in the centrifuged-refed group of rats compared to its corresponding control group. Table 5 shows the decrease in gastrocnemius glycogen of centrifuged rats compared to non-centrifuged controls. Although there appears to be a decrease in muscle glycogen levels during the first hour of centrifugation, these changes were not very significant statistically.

The effects of combined stresses on the glycogen deposition response were followed in rats subjected simultaneously to centrifugation as well as starvation. Results are shown in Fig. 3. Rats subjected to starvation alone show a minimum level of liver glycogen after 24 hr. With continued starvation, there is a progressive increase in liver glycogen which reaches a maximum level on the fourth day. Rats subjected to both starvation and centrifugation, in contrast, show a maximum liver glycogen level after 24 hr which is then rapidly depleted with further exposure. In these animals the amount of glycogen after 3 days of exposure is reduced to an insignificant level. When muscle glycogen of starved-centrifuged rats are compared to corresponding starved control rats, it is found that the 24 hr starved-centrifuged rats have significantly higher amounts of glycogen than corresponding control ($P < 0.001$). By the third day, however, control animals have higher muscle glycogen levels than the starved-centrifuged animals. In general, both liver and muscle glycogen levels of starved-centrifuged rats are reduced after the first day of exposure. Noncentrifuged control rats, in contrast, show a progressive increase in liver glycogen and a concomitant decrease in muscle glycogen with exposure time.

Discussion

These experiments clearly indicate the dependence of the glycogen deposition response on a functionally intact pituitary-adrenal system. This is evident from the observation that adrenalectomy and hypophysectomy abolish the capacity of centrifuged animals to respond. In addition, a correlation can be drawn between the increase in plasma corticosterone levels and the deposition of glycogen in livers of centrifuged animals (cf. Table 3). An exception to this, however, is noted in alloxan-diabetic rats which exhibit a significant increase in plasma corticosterone levels upon centrifugation but with no apparent increase in liver glycogen deposition. A possible explanation for the inability of these animals to respond is that as far as the glycogen deposition response is concerned, adrenal corticosterone may already be exerting an optimal activity and that the additional amounts released during centrifugation stress are without effect. The very high levels of liver glycogen found in these diabetic animals after overnight fasting and the observations that there is adrenal hypertrophy and increased corticosteroid production following alloxan treatment (19, 20) lend support to this view.

Although no measurements were made in this study of the adrenal medullary hormones, there is no doubt that the sympathetic discharge of epinephrine during acceleration stress plays an important role in the mediation of the glycogen deposition response. It has, for example, long been known that administration of epinephrine can effect a "transfer" of glycogen from muscle to liver tissue through the intermediacy of lactate formed from breakdown of muscle glycogen (Cori cycle).

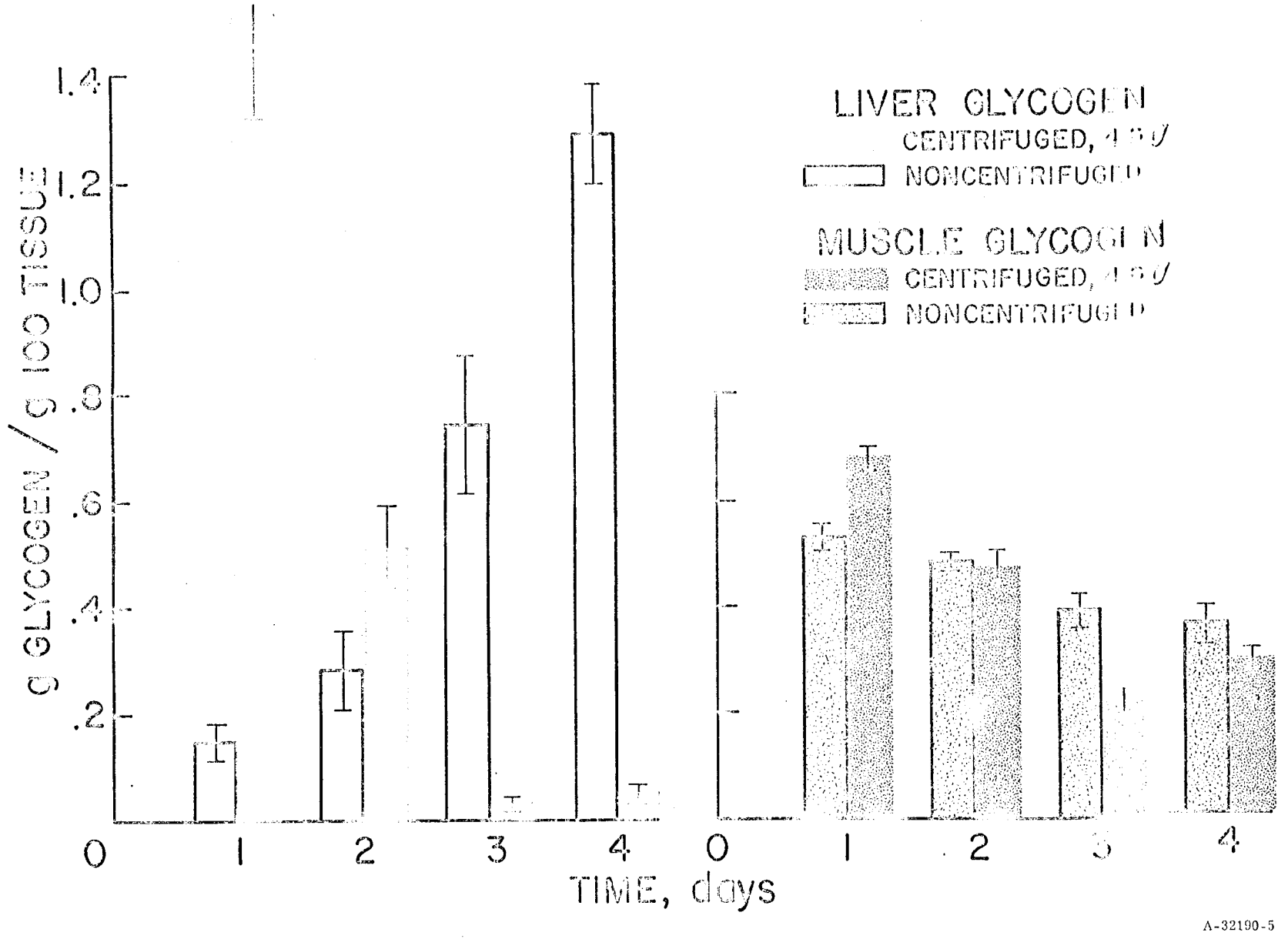


Figure 3

If the decrease in gastrocnemius muscle glycogen (Table 5) is representative of the changes occurring in muscle tissues in general, from considerations of the relative masses of liver and muscle tissues, it is possible to fully account for the glycogen deposited in the livers of centrifuged rats from stress induced muscle glycogenolysis. From the standpoint of essential function in the glycogen deposition response, epinephrine is without question secondary to that of the adrenal corticoids. Long and co-workers (21) have reported that when rats are injected with epinephrine a rapid deposition of liver glycogen occurs, whereas similar treatment of adrenalectomized rats was without effect. Injections of corticoids into adrenalectomized rats resulted in liver glycogen deposition. From these and other considerations, we conclude that while epinephrine may markedly affect the glycogen deposition response the determinant factor is the availability of adrenal corticoids.

The rapid rise in plasma FFA and blood glucose levels in centrifuged rats is probably due to epinephrine action since corticoids are generally known to exert their effects more slowly. Shafrir, et al. (22) have reported on increases in plasma FFA and blood glucose following epinephrine injection of rats. Stressed rats also exhibit similar increases (23).

The decrease in plasma FAA was unexpected since adrenal corticoids have an over-all catabolic effect in terms of protein metabolism and have been reported to cause an increase in plasma FAA when administered to rats (24, 25). However, it has been reported that glucocorticoids stimulate amino acid "trapping" by the liver (26). It is possible that under conditions of acceleration stress physiologic increases in

corticosterone may mobilize plasma FAA to the liver without significantly affecting the catabolism of tissue protein. It is interesting that the amino acids which are preferentially lowered in centrifuged rat plasma are all glucogenic and is suggestive that stimulation of hepatic gluconeogenesis by corticosterone may be a factor in the observed decrease in plasma FAA.

The primary site of action of glucocorticoids on liver glycogen synthesis has not been unequivocally determined. Much progress, however, has been made recently on this fundamental problem. There is increasing evidence to support the postulate that the primary site of action of glucocorticoids is on some rate limiting process of glycogen synthesis lying between glucose-6-phosphate and glycogen (27-30). Studies are currently in progress in this laboratory to delineate this primary site of corticosterone action on acceleration stress induced glycogenesis.

Acknowledgments

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Table 1. Plasma free amino acids of 21 hr fasted female rats centrifuged at 4.5 g for 2.5 hr as measured by ion-exchange chromatography. Values are means of two separate analyses, on a pooled sample from 3 rats.

Amino acid	μmoles per 100 ml plasma Noncentrifuged	Centrifuged
Alanine	26.0	13.7
α-Aminobutyric	1.9	1.8
Arginine	6.9	4.1
Aspartic	0.5	0.2
Citrulline	4.2	3.2
Glutamic	6.1	4.4
Glutamine-asparagine	45.0	37.0
Glycine	13.9	9.9
Histidine	3.3	3.0
Isoleucine	6.3	4.8
Leucine	9.6	7.4
Lysine	31.2	24.7
Methionine	2.8	1.8
1-Methylhistidine	0.8	Trace
3-Methylhistidine	Trace	Trace
Ornithine	3.4	3.6
Phenylalanine-Tyrosine	8.0	5.0
Proline	8.0	5.8
Serine	14.3	10.7
Threonine	13.0	10.2
Tryptophan	3.4	2.8
Valine	11.3	9.0
Total	220	163
Nonamino acids		
Ammonia	16.1	16.6
Ethanolamine	1.1	0.6
Taurine	10.4	8.4
Urea	355	295
Total	383	321

Table 2. Plasma corticosterone of fasted female rats centrifuged at 4.5 g for varying exposure periods measured by the fluorometric and double isotope derivative methods.

Time, hr	Fluorometric μg/100 ml			Double isotope μg/100 ml		
	Noncentrifuged	Centrifuged	Increase	Non-centrifuged	Centrifuged	Increase
0.5	62* ± 14	176 ± 9	114	29*	121	92
1.0	62* ± 14	168 ± 11	106	29*	119	90
2.5	49 ± 13	177 ± 9	128	28	217	189
24	62* ± 14	117 ± 3	55	29*	71	42

Values by the fluorometric method are means ± standard error of 8 rats and are uncorrected for residual plasma fluorescence. Isotope values were obtained on a composite sample from the rats.

*Common control for these series of experiments run on the same day.

Table 3. Comparison of glycogen deposition and plasma corticosterone of normal, adrenodemedullated (ADM), hypophysectomized (HYPOX), adrenalectomized (ADRX) and alloxan-diabetic rats exposed to centrifugation at 4.5 g for 5 hr.

Treatment	No. of rats in each group	Liver glycogen g/100 g tissue			Plasma corticosterone μg/100 ml		
		Control	Centrifuged 4.5 g - 5 hr	Δ	Control	Centrifuged 4.5 g - 5 hr	Δ
Normal	8	0.14 ± 0.04	1.62 ± 0.17	1.48	49 ± 10	106 ± 8	57
ADM	4	0.73 ± 0.04	1.46 ± 0.21	0.73	48 ± 14	131 ± 7	83
HYPOX	7	0.02 ± 0.00	0.11 ± 0.04	0.09	16 ± 1	21 ± 1	5
ADRX	6	0.03 ± 0.01	0.02 ± 0.00	-0.01	8 ± 1	8 ± 2	0
Diabetic	4	1.76 ± 0.59	1.79 ± 0.44	0.03	64 ± 4	110 ± 23	46

Values are means ± standard error of number of animals in each group. Δ = difference, centrifuged minus control. Plasma corticosterone analyses were run fluorometrically and are uncorrected for residual plasma fluorescence. All animals were fasted for 16 hr prior to centrifugation.

Table 4. Liver glycogen of rats centrifuged for 5 hr at 4.5 g immediately after exposure and after 24 hr.

	Glycogen g/100 g tissue		
	Immediately after	24 hr after	
		Fasted	Refed
Noncentrifuged	0.21 ± 0.06	0.71 ± 0.16	8.00 ± 0.25
Centrifuged	1.33 ± 0.12	0.12 ± 0.02	7.48 ± 1.15

Values are means ± standard error of eight animals. Rats were fasted 16 hr prior to centrifugation.

Table 5. Effect of centrifugation on gastrocnemius muscle glycogen.

	Glycogen g/100 g tissue		
Time, hr	Control	Centrifuged 4.5 g	% decrease
0.5	0.50 ± 0.04 (6)	0.40 ± 0.03 (6)	20*
1.0	0.50 ± 0.04 (6)	0.41 ± 0.03 (6)	18*
5.0	0.46 ± 0.08 (4)	0.42 ± 0.06 (7)	8.7

Rats were fasted 16 hr prior to centrifugation.

*Significant $P < 0.1$.

Values are means ± standard error of number of animals in parentheses.

FIGURE LEGENDS

Fig. 1.- Liver glycogen deposition in centrifuged female rats as a function of exposure time. Column heights are means of 20 rats with standard errors shown by the bracketed vertical lines. All rats were fasted for a total of 24 hours including the time of centrifugation. Rats were sacrificed by decapitation.

Fig. 2.- Changes in blood glucose, plasma free fatty acid, and plasma free amino acid nitrogen of centrifuged rats with exposure time. Height of columns are means ± standard error of 6 rats. ☐ solid columns - centrifuged. ☐ light columns - control. Blood samples were obtained from anesthetized rats by heart puncture.

Fig. 3.- Effect of combined stresses of prolonged starvation and centrifugation (4.5 g) on liver and gastrocnemius muscle tissue glycogen. Values are means ± standard error of 6 animals.

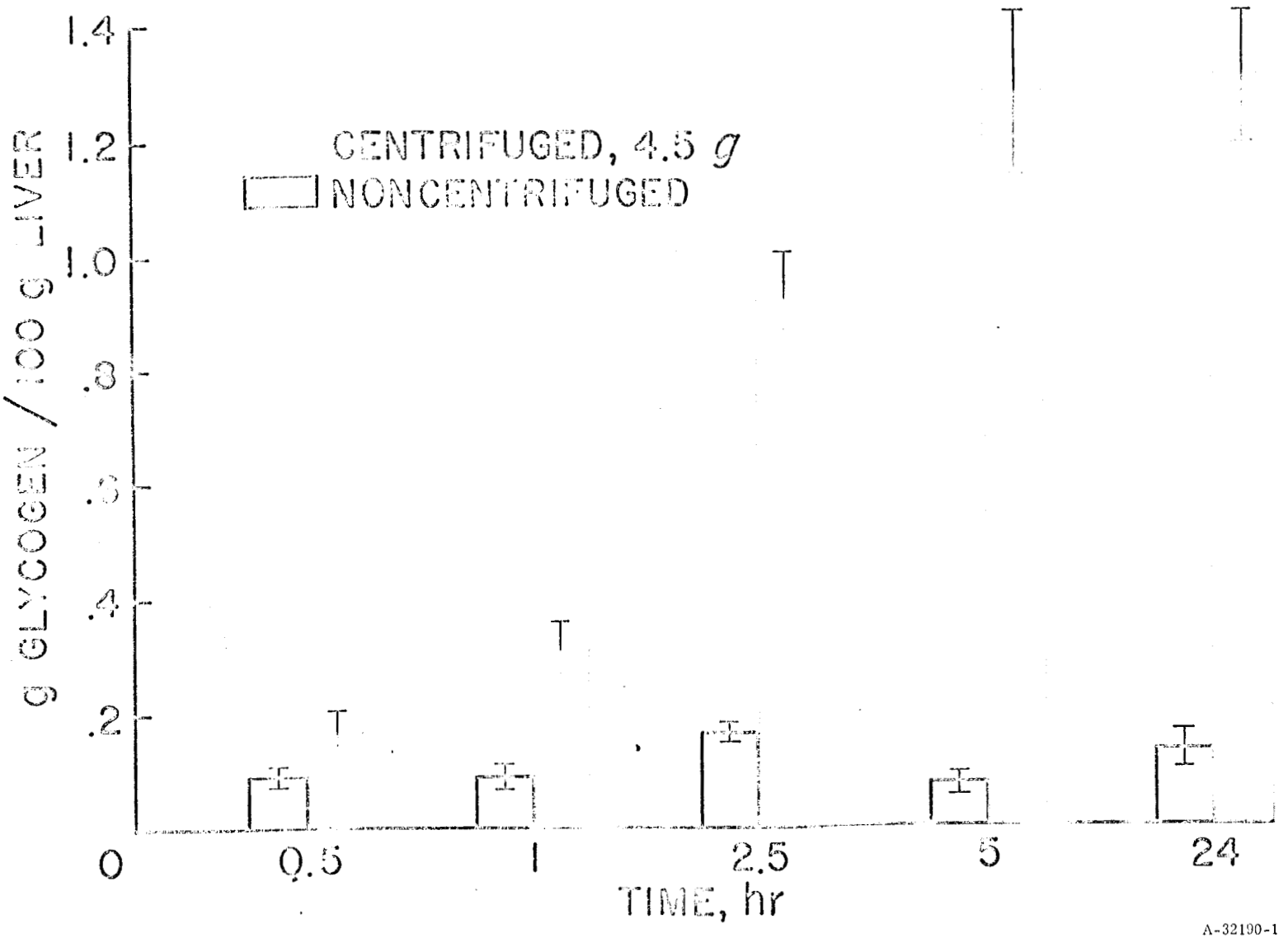

Figure 1

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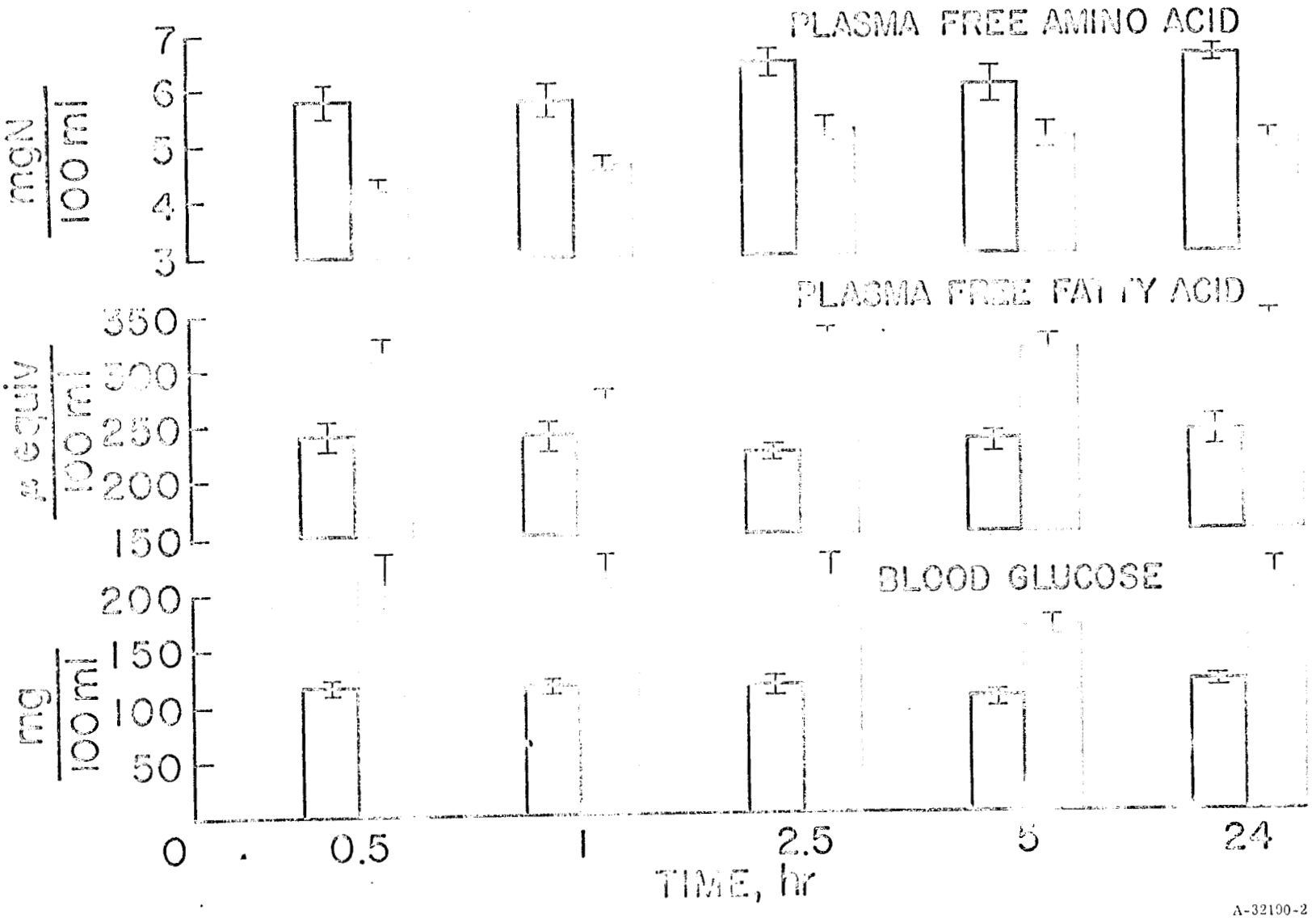

Figure 2

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